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First natural occurrence of the entomopathogenic fungus *Beauveria bassiana* (Bals. Criv.) Vuill. on *Cosmopolites sordidus* (Germar, 1824) (Curculionidae, Coleoptera) in an agroforestry system in the Brazilian Cerrado

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Abstract. The natural occurrence of the fungus *Beauveria bassiana* is an indicator of environmental balance. When the agroforestry system naturally presents this entomopathogenic fungus on the banana weevil *Cosmopolites sordidus*, a pest insect in the banana crops, it reinforces the beneficial effects of this agroecosystem. Thus, this work reports the first natural occurrence of *B. bassiana* on *C. sordidus* for the agroforestry system and the Cerrado biome. The natural occurrence of this parasitic relationship indicates that the agroforestry system may favor the fungal occurrence and spread.

Key words. Banana Weevil, biological control, Cordycipitaceae, pathogenicity

Silva-Neto CM, Calaça FJS, Souza WG, Santos LAC, Pina IJ, Xavier-Santos S, Calil FN (2024) First natural occurrence of the entomopathogenic fungus *Beauveria bassiana* (Bals. Criv.) Vuill. on *Cosmopolites sordidus* (Germar, 1824) (Curculionidae, Coleoptera) in an agroforestry system in the Brazilian Cerrado. Check List 20 (1): 47–52. https://doi.org/10.15560/20.1.47

INTRODUCTION

Cosmopolites sordidus (Germar, 1824), or Banana Weevil (Coleoptera, Curculionidae), is the primary insect pest of Banana crops in South America. The feeding larvae of this beetle create tunnels in the rhizomes and pseudostems of Banana plants, which reduces the water and nutrient uptake due to damage to the vascular system. This causes the death of plants or reduces the size of bunches, which causes a significant loss of this crop in Brazil, especially in areas of the Cerrado (Fancelli et al. 2013).

Control and natural mitigation of *C. sordidus* is encouraged in agroforestry production and aims to use minimal pesticides. The use of biological control by means of entomopathogenic fungi is viable, especially when these fungi naturally occur in the environment. Entomopathogenic fungi are all those that infect insects to use them as hosts to complete part of their life cycle (Mora et al. 2017; Moreira et al. 2019).

Entomopathogenic fungi constitute a phylogenetically diverse group within the kingdom Fungi, and most species belong to the phylum Ascomycota. In the family Cordycipitaceae, *Beauveria* Vuill. represents one of the best-known genera of asexual entomopathogenic fungi and is widely used in the biological control of insect pests, especially its type species, *B. bassiana* (Bals. Criv.) Vuill. (Rehner et al. 2011). Two studies have already shown the effectiveness of using *B. bassiana* in reducing damage to Banana plants caused by *C. sordidus* (Fancelli et al. 2013; Membang et al. 2021). While these studies provide corroboration that this fungus is an excellent biological control of *C. sordidus* in agroforestry systems, the natural occurrence of *B. bassiana* in agroforestry systems in Brazil has not been reported to date. Thus, we report the first natural occurrence of *B. bassiana* in *C. sordidus* in an agroforestry system in the Brazilian Cerrado.

OPEN ACCESS

Academic editor: Roger Fagner Ribeiro Melo
Received: 21 July 2023
Accepted: 2 January 2024
Published: 15 January 2024

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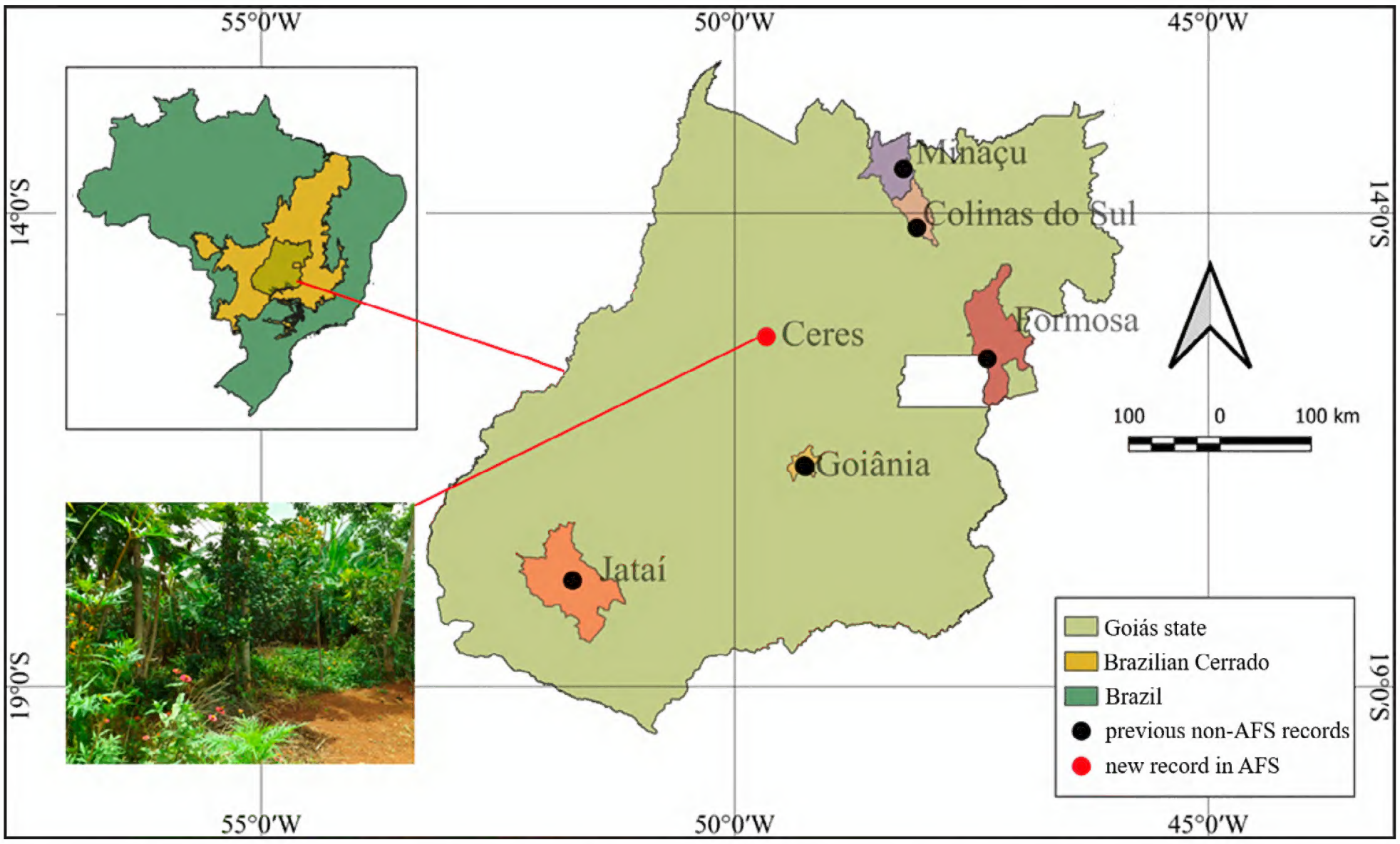

Figure 1. Location map where *Beauveria bassiana* (Bals. Criv.) Vuill was found on *Cosmopolites sordidus* (Germar, 1824) (Curculionidae: Coleoptera) in an agroforestry system in the Brazilian Cerrado.

STUDY AREA

The studied agroforestry system is located within a family property on the outskirts of the municipality of Ceres in the state of Goiás, Brazil (15°18′20.0″S, 049°39′58.5″W; Figure 1). The property is 1 ha in area and was planted in 2015. The climate type is rainy tropical [Aw (tropical savanna) and Cwa (temperate rainy dry winter)], according to the Köppen classification (Pecl et al., 2017) Native and exotic tree species present are pequi (*Caryocar brasiliense* Cambess.), baru (*Dipteryx alata* Vogel), jatobá (*Hymenaea* L. sp.), among others. Planted trees for agriculture include acerola, banana, sweet potato, cocoa, coffee, cashew, yam, citrus, papaya. The Banana crop, which is the main produce of this property, consists of *Musa* sp. (apple variety, unknown genotype). The fruit grow in clumps of about 50, and each clump has at least 3–5 pseudostems.

METHODS

In September 2018, during the cutting of pseudostems of a banana plant, an adult corpse of an insect infected by a filamentous fungus was found. The corpse was collected and sent for identification. To analyse the fungus and confirm its identification, fragments of the fungus were removed using a sterile needle. Semi-permanent slides were prepared of these fragments, using polyvinyl alcohol-lactic acid-glycerol (PVLG) for fixation and lactophenol cotton blue for staining. Microscopic observations were made using an Olympus CX31 optical microscope and attached digital camera. Macroscopic observations were made using a Leica EZ4 stereomicroscope and the Leica Application Suite Leica image capture system (Leica Microsystems). Conidia measurements were taken using Piximètre version 5.9 R 1532 (Henriot and Cheype 2017). For the measurements, Q refers to the quotient between the length and width, Qm is the medium value of Q, and *n* is the number of measured conidia. Color names and codes used in the macroscopic descriptions are based on Kornerup and Wanscher (1978).

The fungus was isolated on potato dextrose agar culture medium (PDA, 3.9 g in 100 mL of water) with the addition of 0.25 mg/mL of chloramphenicol in a Petri dish and incubated in BOD at 26 °C. After the development of the colonies, the cultures were grown in PDA. For phylogenetic analyses, approximately 25 mg of frozen hyphae from the PDA cultures were crushed in a mortar under liquid nitrogen, and the total genomic DNA was extracted and processed further, as described by Lopes et al. (2013). Phylogenetic identification was performed by amplifying the B locus intergenic region (Bloc), and the primer pair B5.1F and B3.1R was used for both PCR and sequencing, as described by Rehner et al. (2011). Sequencing was performed by Macrogen Inc. (Seoul, South Korea) and edited using DNA baser (DNABaser Sequence Assembler version 3, Heracle Biosoft, Pitesti, Romania). Consensus sequences were aligned using MEGA version 5.03

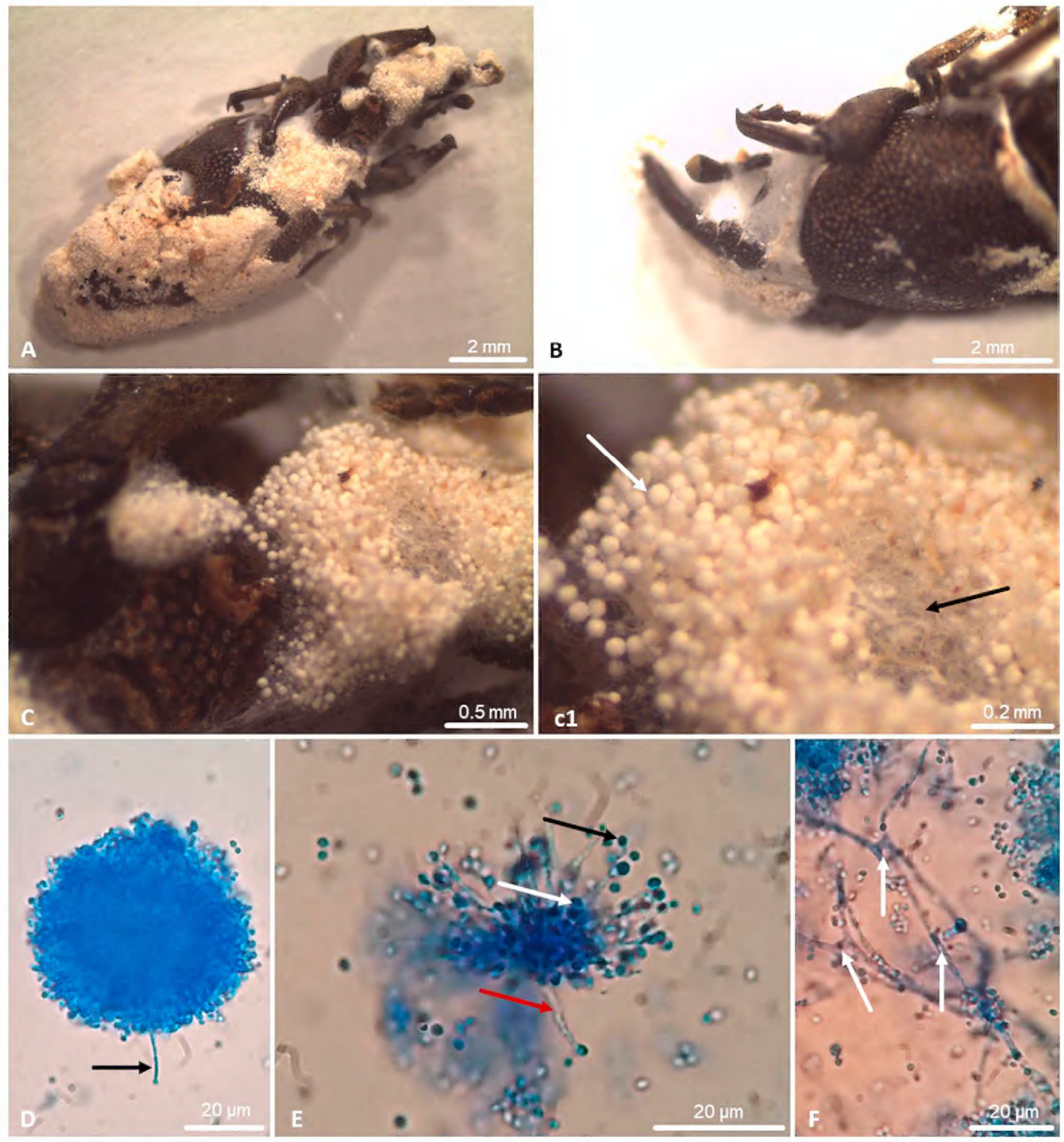

Figure 2. *Cosmopolites sordidus* corpse infected by the entomopathogenic fungus *Beauveria bassiana*. **A.** Insect with symptoms of infection. **B.** Detail of the places where the aerial mycelium and conidiophores emerged from the beetle. **C, C1.** detail of the conidiophores (white arrow) and the mycelium covering the insect (black arrow). **D.** A conidiophore, composed of clusters of conidiogenic hyphae; arrow indicates sustaining hypha. **E.** A cluster of conidiogenic hyphae and conidia; black arrow indicates young conidia produced at apex of hyphae; white arrow indicates older conidia. Red arrow points to the rachis of a conidiogenic hyphae, where scars of conidia already released are visible. **F.** Septate and branched hyphae (arrows).

(Tamura et al. 2011) with Clustal W, and the final alignments were adjusted manually. The matrix dataset was comprised of 29 sequences with sequence sizes of 1633 bp, and the reference sequences were from the ex-type strains. Analysis of the alignment was made using maximum-likelihood and Bayesian analyses.

MrBayes version 3.1.2 was used for the Bayesian phylogenetic analysis and PAUP version 4.0 for the maximum-likelihood analysis (ML). The AIC as implemented in MrModelTest version 2.2 with the GTR + I + G model selected for both analyses (Ronquist et al. 2012). Nodal support was assessed for the ML analysis with nonparametric bootstrap using 1,000 pseudoreplicates. Bayesian analysis was run over ten million generations, with tree sampling every 100 generations. The first 25% of trees were discarded prior to consensus tree calculation. Bayesian prior probability and percentage of bootstrap support derived from ML analysis were calculated.

Vouchers of the colonized insect and cultures were deposited in the mycological collection of the Herbarium of the Universidade Estadual de Goiás (HUEG 12001) and the cultures were vouchered in the Laboratory of Basic and Applied Mycology and Scientific Dissemination (FungiLab) of the same institution (SXS 640).

RESULTS

New records. BRAZIL – **Goiás** • agroforestry system located on the outskirts of the municipality of Ceres; 15°18′20.0″S, 049°39′58.5″W; 600 m elev.; 21.IV.2018; W.G. Souza leg.; manual collection; HUEG 12001, SXS 640 (Figure 1).

Identification. Macroscopically, *Beauveria bassiana* presents white (1A1) to yellowish white (1A2) colonies with a woolly to cottony appearance. Colonies are composed of aerial hyphae and aggregates of conidiogenic hyphae and conidia, which form spherical clusters (conidiophores) 50–70 µm in diameter (Figure 2A–C).

Microscopically, the fungus has hyaline, septate, and very branched hyphae with smooth walls, measuring up to 2.5 µm thick. Conidiogenic hyphae are either in groups or single and have an ampulliform basis.

The rachis is 10–20 µm long and with several conidial scars, which have a zigzag appearance. Conidia (1.7) 1.9–2.7 (2.8) × (1.2) 1.4–1.8 (2.1) µm [Q = 1.2–1.7; Qe = 1.4; *n* = 30] are hyaline, globose to subglobose, and not septate; they have thin, smooth walls (Figure 2D–F).

The isolate was identified as *Beauveria bassiana* (s.l.), both by its morphological and molecular characteristics. It has high genetic similarity to other isolates of this species. The cladogram, which includes strains of different species of *Beauveria*, shows that our isolate (SXS 640) groups with *B. bassiana*. In fact, it shows that SXS 640 has greatest similarity to the isolates CG425 (isolated from *Rhammatocerus schistocercoides* Rehn, 1906 (Orthoptera, Acrididae) from Mato Grosso, Brazil) and ARSEF 1829 (isolated from *Castnia licus* Drury, 1770 (Lepidoptera, Castniidae) from Pernambuco) (Figure 3).

DISCUSSION

Our infected insect specimen (Figure 3A–C) showed complete development of the fungus. The final stage of the infection process consists of the saprophytic growth of the fungal mycelium in the dead body of the insect. There is subsequently massive production of new conidia in the conidiophores (Figure 3C, C1), and the fungus is able to infect new individuals at this stage. The process of infection and growth of the fungi in the insect includes adhesion of the conidia to the insect cuticle, germination, penetration through the cuticle, responses to the host defense mechanisms (immune response), growth, and proliferation. Once inside the host, it finally causes the death of the insect (Mora et al. 2017).

Our report of *B. bassiana* parasitic on *C. sordidus* is the first from an agroforestry system. in the Cerrado. Our observations of *B. bassiana* in the study area suggests that the population of *C. sordidus* in that plantation may be under natural control. Our discovery of the natural occurrence of *B. bassiana* in insect pests in agroforestry systems is of importance in that strains of the fungus more adapted to the conditions of the Cerrado and in agroforestry systems, could be studied further and applied as biological control to agricultural crops. Our specimen of *B. bassiana* had many conidiophores, which is evidence that this fungus is dispersing and causing new infections, and we note that this environment appears favorable for the propagation of this beneficial fungus in the agroecosystem.

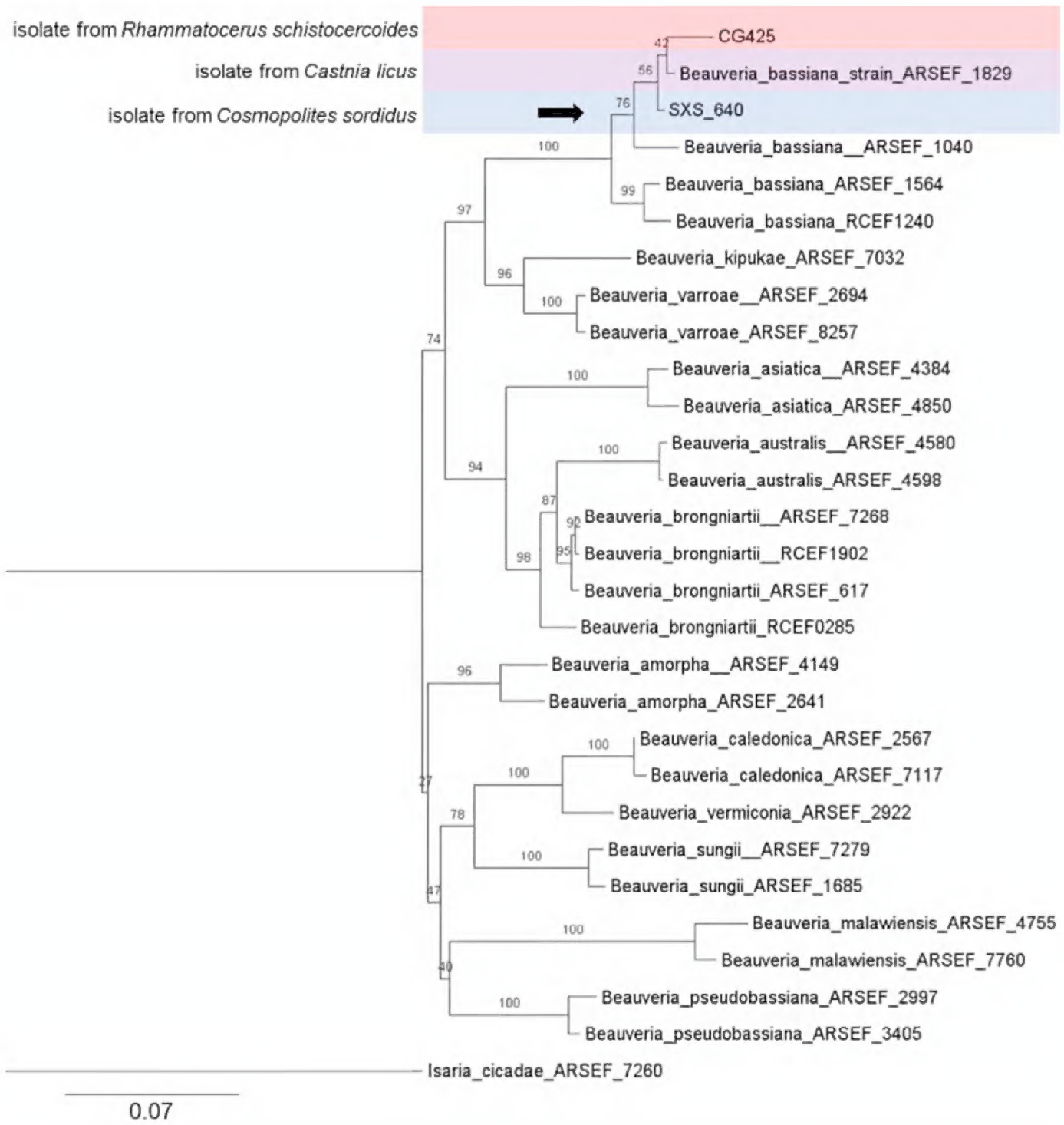

Figure 3. Phylogram of aligned bloc-gene sequence data for *Beauveria* strains. Strain with code SXS 640 (arrow) is the newly isolated sample. It is coupled with the CG code from the Invertebrate Fungal Collection at Embrapa Genetic Resources and Biotechnology (Brasília, Federal District, Brazil) and with the ARSEF code are from the Agricultural Research Service Collection of Entomopathogenic Fungal Cultures (US Department of Agriculture).

ACKNOWLEDGEMENTS

The first author thanks CNPq for financial support and the funding agencies of Call N 21/2016, namely, MAPA, MCTIC, MEC, and SEAD - Casa Civil. We also thank the Universidade Estadual de Goiás and the Graduate Program in Cerrado Natural Resources, the family of farmers (Janaina and family) who own the land on which our study initiated and who welcomed us into their homes with such receptivity. We also thank the anonymous reviewers and the academic editor for their contributions, suggestions, edits and comments that contributed greatly to improving the final quality of the manuscript.

ADDITIONAL INFORMATION

Conflict of interest

The authors declare that no competing interests exist.

Ethical statement

No ethical statement is reported.

Funding

This study was financially supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the funding agencies namely: MAPA, MCTIC, MEC and SEAD - Casa Civil [Call nº 21/2016].

Author contributions

Conceptualization: CMSN, FJSC, SXS, FNC. Data curation: WGS, LACS, IJP. Formal analysis: WGS, LACS, IJP. Investigation: CMSN, FJSC, SXS, FNC. Methodology: CMSN, FJSC, WGS, LACS, IJP, SXS. Project administration: CMSN, FJSC. Resources: CMSN, FJSC, WGS, LACS, IJP, SXS. Software: FJSC. Supervision: CMSN. Validation: CMSN, FJSC. Visualization: FJSC. Writing – original draft: CMSN, FJSC, SXS, FNC. Writing – review and editing: CMSN, FJSC, WGS, LACS, IJP, SXS, FNC.

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Data availability

All data that support the findings of this study are available in the main text.

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